	R_1	R_2	R_3	R_4
Picrodendrin-A:	H	OH	CH_2OH	OCH_3
-M:	OH	H	CH_3	OCH_3
-Q:	H	H	CH_3	OCH_3
-T:	H	OH	OCH_3	CH_2OH

	R_5	R_6	R_7	R_8	R_9
Picrodendrin-E:	OH	H	OH	H	OH
-F:	H	OH	OH	OH	OH
-L:	H	OH	H	OH	OH
-O:	H	H	OH	H	OH

Figure 1. Structures of picrodendrins.

Keywords: picrodendrin; terpenoid; structure–activity relationship; ionotropic GABA receptor; noncompetitive antagonist; binding site

Naturally occurring, biologically active compounds provide valuable tools for elucidating the molecular basis of physiological events. In the present study, 28 picrotoxane terpenoids, including picrodendrins (Fig 1)[1,2] isolated from the Euphorbiaceae plant, *Picrodendron baccatum* (L) Krug & Urban, have been evaluated for their ability to inhibit specific binding of [^{3}H]1-(4-ethynylphenyl)-4-propyl-2,6,7-trioxabicyclo[2.2.2.]octane (EBOB), the noncompetitive antagonist of ionotropic GABA receptors, to rat-brain and housefly-head membranes.[3] Picrodendrin Q was the most potent competitive inhibitor, with IC_{50} values of 16nM (rat) and 22nM (houseflies). The spiro γ-butyrolactone moiety, containing a carbonyl group conjugated with an unsaturated bond at the 13-position and the hydrophobic substituents at the 4-position play important roles in the interaction of picrodendrins with their binding site in rat GABA receptors. In contrast, such structural features are not strictly required in the case of the interaction with housefly GABA receptors; the spiro γ-butyrolactone, bearing the 16-sp^3 carbon atom at the 13-position and hydroxyl groups at various positions are somewhat tolerated.

Quantitative structure–activity studies have clearly shown that the electronegativity of the 16-carbon atom and the presence or absence of the 4- and 8-hydroxyl groups are important determinants of potency of norditerpenes in housefly receptors, while the negative charge on the 17-carbonyl oxygen atom is likely to be important in the case of rat receptors. These findings are consistent with those of our previous studies[4] that there are significant differences in the structures of their binding site between rat and housefly GABA receptors.

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Insecticidal toxins from the bacterium *Photorhabdus luminescens*: gene cloning and toxin histopathology

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Abstract: Four toxin complexes, Tca, Tcb, Tcc and Tcd from the culture broth of *Photorhabdus luminescens* have been purified and the four toxin complex encoding loci, *tca, tcb, tcc* and *tcd*, cloned. Genetic knockout of either *tca* or *tcd* reduced oral toxicity to *Manduca sexta*, and knockout of both loci eliminated activity. Purified Tca specifically affected the insect midgut, despite its putative normal delivery directly into the insect haemocel. These *Photorhabdus* toxins may form useful alternatives to other orally active bacterial protein toxins such as those from *Bacillus thuringiensis*.

Keywords: *Photorhabdus luminescens*; bacterial toxins; toxin complexes; insecticidal activity

Photorhabdus luminescens (Enterobacteriaceae) inhabits

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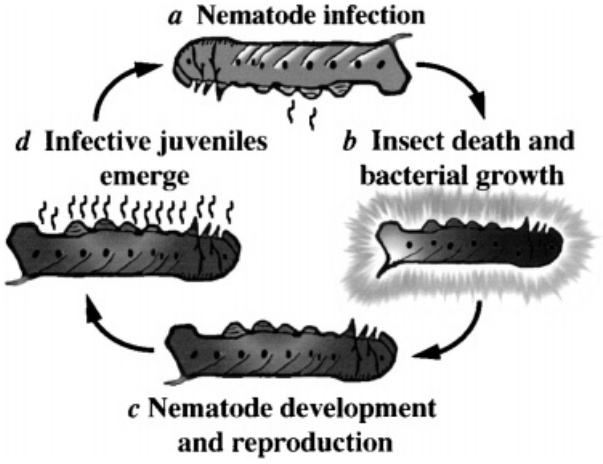

Figure 1. Life cycle of the *Photorhabdus luminescens* bacterium which lives in a mutualistic association with entomophagous nematodes. (a) Release of the bacteria from the gut of the invading nematode, (b) death of the host and (c) nematode replication in the insect cadaver. At this stage the bacteria cause the insect cadaver to emit light (the biological role of which is uncertain). (d) Infective juvenile nematodes are then released from the cadaver for re-infection of other insects.

the gut of entomopathogenic nematodes of the family Heterorhabdititae.[1] Following invasion of an insect by the nematode, *P luminescens* are released into the insect haemocoel. The bacteria and nematodes then replicate within the insect cadaver.[2] At this stage the bacteria emit light causing the cadaver to glow. After several rounds of reproduction in which the nematodes feed off both the bacteria and the insect carcass, infective juveniles emerge to colonise new hosts (Fig 1).

P luminescens can be readily cultured away from its host and a few bacterial cells can kill a single insect.[3] The work of others had suggested that insecticidal activity was associated with a range of different compounds including proteases, lipases and lipopolysaccharides.[4–7] However, previous purification work had shown that insecticidal activity was associated with the high-molecular-mass fraction of the culture broth.[8] Following further purification and a final high-performance liquid chromatography (HPLC) step, four high-molecular-mass toxin complexes can be resolved from the orally toxic fraction, termed toxin complexes A, B, C and D (or Tca, Tcb, Tcc and Tcd). Individual toxin complexes migrate as single (or double) components on native gels, but can each be resolved into a number of different polypeptides by SDS-PAGE.[9]

In order to characterise further the composition of each of the toxin complexes we raised both a polyclonal and a monoclonal antiserum against the high-molecular-mass toxin fraction which contains all four toxin complexes. We then screened a *P luminescens* genomic library with both antisera. The antisera recognised clones expressing components of four different *toxin complex (tc)* encoding loci, termed *tca, tcb, tcc* and *tcd*. Comparison of *N*-terminal protein sequences derived from purified polypeptides in the native broth with the predicted amino acid sequences of the *tc* loci confirmed that *tca, tcb, tcc* and *tcd* encode the proteins Tca, Tcb, Tcc and Tcd respectively. The sequences of these genes have been reported elsewhere.[9] The predicted amino acid sequences of the four *tc* loci have little, if any, similarity to other known protein toxins. However, short stretches of both Tca and Tcc share similarity with *Salmonella* plasmid virulence factors B and A respectively (termed spvB and spvA). These virulence factors are responsible for the ability of certain *Salmonella* strains to replicate in monocyte-derived macrophages, and suggest a possible role for the *P luminescens* homologs in overcoming insect haemocytes.

Despite our ability to reconstitute antigenicity, the toxin complexes are not exported from *E coli* and the pattern of apparent protease cleavage seen in the *P luminescens* broth is also not reproduced. Therefore in order to confirm the nature of these complexes as orally active toxins we used two approaches. First, we purified sufficient quantities of Tca to perform LD_{50} determinations on neonate *Manduca sexta* Joh exposed to toxin added topically to artificial diet. Tca is orally active in the ng cm^{-2} range, which is equivalent to that of some *Bacillus thuringiensis* Berliner δ-endotoxins.[10] Second, we knocked out each of the *tc* loci in the same

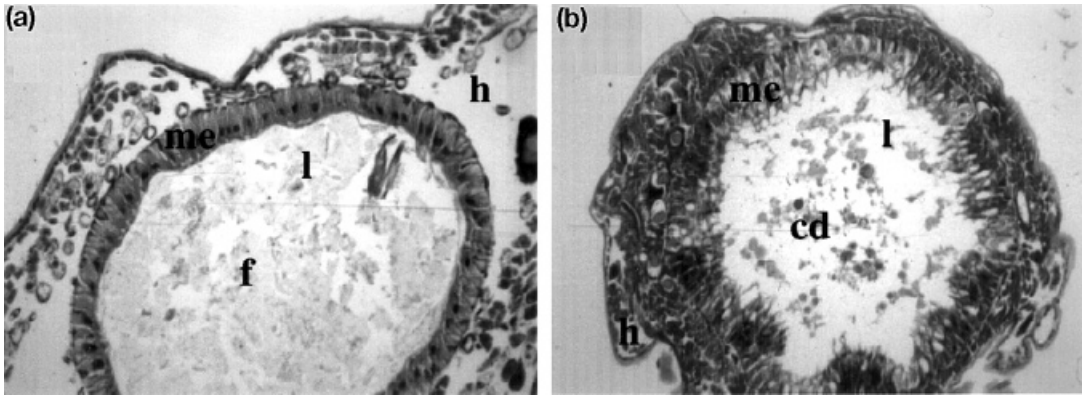

Figure 2. Histopathology of purified toxin complex A (Tca) on the midgut of *Manduca sexta*. (a) Normal cross-section of midgut showing: the intact midgut epithelium (me), the gut lumen (l) and the food (f) within it. Note the extent of the surrounding haemocoel. (b) Cross-section of midgut after ingestion of Tca-treated diet. Note the deposition of cellular debris (cd) formed from the blebbing of the midgut epithelium into the lumen. Note also the reduction in the volume of the haemocoel as feeding ceases and the insect starts to dehydrate.

strain of *P luminescens* (W14) and then tested the effect of the mutant bacterial broths in our oral bioassay. Deletion of either *tca* or *tcd* individually (as tca^- or tcd^- mutant strains) greatly reduced the oral toxicity of the broth to *M sexta*, whereas deletion of both *tca* and *tcd* together (in the tca^-/tcd^- double mutant) eliminated oral toxicity altogether. These results suggest that both Tca and Tcd are involved in oral toxicity to Lepidoptera. However, we have been unable to purify sufficient quantities of Tcd to perform an LD_{50} determination.

In order to examine the effects of Tca on the lepidopteran gut and compare it to that previously documented for both the *B thuringiensis* δ-endotoxins and vegetative insecticidal proteins (Vips), and for cholesterol oxidase,[11–15] we sectioned *M sexta* neonates at intervals after oral ingestion of toxin. After several hours, toxin-treated midguts showed an accelerated rate of epithelial blebbing (Fig 2). This blebbing of the midgut epithelium into the lumen continues until the basement membrane is exposed and the epithelium is essentially destroyed. Both the columnar cells and the goblet cells appear to be attacked. Interestingly, a similar histopathology can be observed following injection of Tca directly into the insect haemocoel, which is presumably the normal route of delivery of the toxin by the bacterium.[16]

In conclusion, we have purified four toxin complexes from the culture broth of *P luminescens* and cloned the four toxin complex-encoding loci. Genetic knockout of either *tca* or *tcd* reduces oral toxicity to *M sexta* and knockout of both loci eliminates activity. Purified Tca shows effects specifically on the insect midgut, despite its putative normal delivery directly into the insect haemocoel. These *Photorhabdus* toxins (Phts) may form useful alternatives to other orally active bacterial protein toxins such as those from *B thuringiensis* (Bt).

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Synthesis of a biotin-like phosphonate model compound for (+)-hydantocidin

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Abstract: Approaches to the synthesis of a biotin-like phosphonate are described. It was hoped that this would be a simpler model compound for the

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